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Characterisation of a Panel of Monoclonal Antibodies Raised Against Recombinant HCV Core Protein

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Hepatitis C virus (HCV) has as yet no practical culture system so any antigen or antibody studies must be carried out using recombinant antigens. In this study, HCV core sequence was amplified by PCR, inserted into pRSET, and expressed in *E. coli*. The resultant core protein was purified using nickel affinity chromatography which bound the six histidine tag attached to the N-terminus of the protein. After elution in imidazole buffer, the core protein was used to immunise Balb/c mice and monoclonal antibodies against HCV core were raised. Six monoclonals were examined in a variety of assays. All of them recognised the p27 kDa protein which they were raised against and 2D2 and 3D7 recognised the core component of an HCV Recombinant Immunoblot Assay (RIBA). None of the antibodies recognised the linear peptides in an Innolia HCV assay. 2D2 showed cytoplasmic granular staining in 1–5% of cells in frozen sections of HCV infected livers. Cross-competition assays between themselves and human anti-HCV core positive sera divided the antibodies into two main groups (I and II), with a sub-division of group I into a and b. Group I antibodies were unable to be inhibited by human anti-HCV sera whereas group II antibodies were inhibited by these sera (up to 62%). Epitopes recognised by all the monoclonals were probably conformational with the group I epitope being located within the first 105 amino acids of the core sequence and the group II epitope between amino acids 105 and 160.

KEY WORDS: HCV core expression, anti-HCV core monoclonals

INTRODUCTION

The hepatitis C virus (HCV) is a major cause of viral hepatitis. It affects over 1 million individuals causing a range of conditions from chronic hepatitis to hepatocellular carcinoma [Duskeiko, 1992]. HCV has a positive stranded RNA genome which is 9.4 kb in length and it is considered to be related to flavi and pestiviruses on the basis of the genome organisation [Miller and Purcell, 1990].

Viral structural proteins are encoded by a single continuous polyprotein sequence at the 5′ end of the genome. Two domains have been identified in the HCV structural polyprotein [Takeuchi et al., 1990a], firstly the core or nucleocapsid region (amino acids 1–192) and secondly, the envelope protein (amino acids 193–810). The core sequence has been shown to have a high degree of homology between different strains of the virus [Takeuchi et al., 1990b]. A number of putative immunodominant regions in highly conserved regions of the core have been identified using fusion proteins [Nasoff et al., 1991].

Since "native" HCV proteins from cell culture are unavailable, serologically based diagnostic assays such as ELISA or RIBA rely upon the use of recombinant HCV proteins, expressed in bacterial (*Escherichia coli*) cells or in a eukaryotic system. Also, oligopeptides covering regions predicted to be immunogenic have been incorporated into some assays. The ability of these proteins to mimic the "native" proteins may be questionable.

Although several groups have reported expression of the 22kDa core protein in *E. coli* or a mammalian cell system [Handschuh and Caselmann, 1995; Milton et al., 1995; Harada et al., 1991], so far none have reported the generation of a panel of monospecific antibodies using the whole 22kDa core protein as the immunogen. In this study, monoclonal antibodies with anti-HCV core specificity were produced and characterised. They were also used for blocking experiments with anti-HCV sera, in epitope mapping (pepscan) and as detection reagents in cryostat liver section immunohistology (IH).

METHODS

Isolation of HCV Core RNA From Serum

Serum from a patient with high titre HCV RNA (genotype 1b, Inno-lipa, Innogenetics, Antwerp, Belgium) was used to amplify cDNA from the core region of the virus. RNA isolation was carried out according to the Chomc-

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zynski and Saachi [1987] method. cDNA synthesis and PCR were carried out according to the methods of Garson et al. [1990] with two rounds of amplification using primers detailed below; sense 322# 5′ GACGGATCCC-CATGGGCACGAATCC and anti-sense 950# 5′ GCT TGAATTCGAGCAATCGTTCGTG and the 322# with anti-sense 907# 5′ GCTTCCGCTTATGAATTCCG-CAACG for the second round.

Cloning and Expression

The 600 base pair (bp) product was inserted into the TA cloning vector pCRII (Invitrogen, Leek, Netherlands) according to the manufacturer's instructions (indeed, all kits mentioned in this paper were used according to the manufacturers instructions). The HCV core sequence was then digested out from the pCRII with *BamH1* and *EcoRI* and inserted into pRSET C (Invitrogen). The core protein was then expressed in *E. coli* JM109DE3 cells. Cell lysates were prepared by adding *10 ml* of buffer A (8M Urea, 0.1M NaH_2PO_4, 0.5M NaCl, 0.01M Tris pH 8.0) per gram of cell pellet and then the crude lysate was sonicated on ice for three 10 second bursts at a medium intensity setting, then flash frozen in liquid nitrogen. After thawing the lysate at 37°C, the sonication freeze thawing cycle was repeated twice more before storage at −20°C. A plasmid expressing the chloramphenicol acetyl transferase protein (CAT, Invitrogen), was used to control for expression. This produced a protein of 28 kDa.

Purification of Core Protein

The pRSET vector was designed to add on an N-terminal metal binding domain to the secreted protein. This enabled the core protein to be purified by immobilised metal affinity chromatography (IMAC). The manufacturers (Invitrogen) recommended several different purification methods, both native and denaturing. A denaturing protocol was followed, using column chromatography and 300 mM imidazole, to elute the bound core protein [Hoffman and Roeder, 1991; Janknecht et al., 1991]. The fractions containing the core protein were dialysed overnight at 4°C in a "refolding" buffer containing 0.5M Urea and 0.05M Tris/HCl pH 8.0 and then concentrated by dialysis against sucrose.

ELISA for Detection of HCV Core Antigen

To demonstrate HCV core immuno-reactivity an ELISA was developed. A 1:800 dilution of an ammonium sulphate precipitate of a rabbit serum containing antibodies to the HCV core region amino acids 29–45 was used to coat the solid-phase (maxi-sorb plates, Nunc, Gibco BRL, Paisley, Scotland). This rabbit serum was raised against an oligopeptide and was a gift from B. Rodger (Murex Diagnostics, Dartford, Kent, UK). Fractions containing HCV core protein were titrated in phosphate buffered saline (PBS) containing 0.05% Tween 20 (T), 1% bovine serum albumin (BSA) and 0.07% sodium dodecylsulphate (SDS) and added to the rabbit anti-HCV core coated plates. Baculovirus expressed HCV core (BHC15 fusion protein) which contained the first 141 amino acids of HCV attached to the polyhedrin protein (46% and 54% respectively), also a gift from B. Rodgers, was used as a positive control for the assay. The antigens were incubated at 37°C for 1 hour and then the plates were washed and a high-titre polyclonal human anti-HCV core serum (291) which had been conjugated to horseradish peroxidase (HRPO) was added. A TMB (3,3′, 5,5′-tetramethyl benzidine) substrate was used to detect the HRPO activity and the optical density (OD) values at 450 nm were recorded.

Western Blot

Samples of the expressed core were run on 15% polyacrylamide gels (SDS PAGE) in a Biorad Mini Protean II System (Hemel Hempstead, Herts, UK). A Horizbot Blotter (Genetic Research Instruments, Felsted, Essex, UK) was used to transfer to proteins from the SDS PAGE gels to nitrocellulose in a semi-dry system. CAT expressed protein and BHC 15 fusion protein were chosen as negative and positive control samples. The antigens were detected using the HRPO conjugated human anti-HCV core serum 291, and a 4-chloro-1- naphthol substrate.

Immunisation and Fusion

8-week-old female Balb/c mice were immunised subcutaneously in the neck with 20 μg of semi-purified core protein. This had been mixed with an equal volume of Titrermax adjuvant (25 μl:25 μl, Stratech Scientific Ltd, Luton, Beds, UK). After two subcutaneous boosts with 10 μg HCV core/Titermax approximately one month apart, an intra-peritoneal injection of 10 μg of HCV core alone was given. A final tail vein injection of 5 μg was given 2 weeks later. Fusion of the mouse spleen cells with the JKAg myeloma cells took place 3 days after the tail vein injection. The fusion was carried out according to the protocol described by Tedder et al. [1982].

Screening Monoclonals for Anti-HCV Core Activity

Anti-core activity was determined by a direct binding ELISA. The BHC15 fusion protein was coated onto the solid phase in carbonate/bicarbonate buffer at 0.5 μg/ml. After quenching (PBS containing 1% BSA), supernatant fluid (SNF) from the hybridomas was added to the BHC15 coated plates at a 1:4 dilution in PBST BSA (1%) and incubated at 37°C for 1 hour. After washing the plates, an anti-mouse HRPO conjugate (P260, Dako Ltd, High Wycombe, Bucks, UK) was added for 1 hour and then TMB substrate added. Hybridomas which gave OD_{450} of above 0.5 were considered positive. Six hybridomas were cloned and SNF or ascitic fluid was collected [Tedder et al., 1982]. The hybridomas were also screened in a Murex HCV ELISA where the kit anti-human conjugate was replaced with the P260 anti-mouse conjugate.

Analysis of Anti-HCV Core Monoclonals

The antibodies were isotyped (Isotyping kit, Serotec Ltd, Oxford, UK) and the ascitic fluid analysed by serum protein electrophoresis (SPE, Beckman Instruments,

High Wycombe, Bucks, UK). The antibodies (IgG1, IgG2a, IgM, and IgA) were purified from the ascitic fluid by either ion exchange chromatography (DE52, Whatman Ltd, Maidstone, Kent UK) [Tedder et al., 1982], protein A or by Sephacryl S300 chromotography (Pharmacia) [Hudson and Hay, 1989]. The antibodies were used in the following assays.

Immunoblotting Using Monoclonal Anti-HCV Core SNF's

Blotting was carried out according to the manufacturer's instructions for the Inno-lia HCV III kit (Innogenetics) and the Murex HCV RIBA. However the anti-human kit conjugates were replaced with an anti-mouse conjugate (P260). The BHC15 fusion protein and the core protein were analysed by Western blotting. The proteins were loaded on the SDS PAGE gels and then transferred to the nitrocellulose. Neat SNF from the monoclonals was then added to the nitrocellulose. The anti-mouse HRPO conjugate (P260) was added to detect the binding and 4 chloro-1-naphthol used as the substrate.

Cross-Blocking Immunoassays

Nunc maxi-sorb plates, coated with BHC 15 fusion protein, were used to compete 50 μl HRPO labelled human serum 291 1:200 (anti-HCV core) with 50 μl of monoclonal immunoglobulin (5 μg/50 μl). After incubation at 37°C for 1 hour, the binding of HRPO was detected with TMB substrate. The immunoglobulin fraction of the monoclonals was also I^{125} radiolabelled [Salcinski et al., 1979] and cold unlabelled immunoglobulin (5 μg/50 μl) from the monoclonals and several anti-HCV positive sera (neat), were allowed to compete for binding to the BHC15 coated plates, at a 1000 molar excess over the radiolabelled monoclonals (50 μl, 50,000 cpm).

Immunofluorescence

Immunofluorescence was carried out using an equal mixture of baculovirus cells, secreting the BHC15 core fusion protein and baculovirus negative control cells, acetone fixed onto slides (a gift from B. Rodgers). 25 μl of each monoclonal SNF (neat) was added to the slides. An anti-HIV *gag* monoclonal was used as a negative SNF [Ferns et al., 1987]. After 40 min at room temperature the slides were washed with PBS and an anti-mouse fluorescein conjugate (Dako Ltd, 1/40) added. The slides were left for 20 min before washing and then mounted in 50% glycerol. Fluorescence was scored as either positive or negative.

Immunohistology

Two anti-HCV monoclonals were tested for their ability to detect HCV core antigen in liver tissue by the immunoalkaline phosphatase technique [Naoumov et al., 1990]. Cryostat liver sections, from HCV infected patients, were fixed with acetone/chloroform 1:1 for 5 minutes. After blocking non-specific absorption with 10% normal goat serum in Tris-buffered saline (pH 7.4), the sections were incubated with the monoclonal SNF overnight at 4°C. Alkaline phosphatase labelled, rabbit anti-mouse antibody and alkaline phosphatase-anti-alkaline phosphatase complexes, APAAP (all from Dako Ltd), were applied consecutively on the sections to demonstrate the binding of the monoclonal antibodies. The alkaline phosphatase reaction was developed using a naphthol buffer and Fast Red TR salt. The sections were counterstained with haematoxylin. Liver tissues from HCV negative patients with other forms of hepatitis and a normal liver were used as negative controls.

TABLE I. ELISA Results From a Typical Purification of HCV Core *E. coli* Lysates

	Dilution of HCV core lysate	
	1:100	1:1000
Before adding to Ni column	2.615*	2:095
40mM imidazole fraction 1	1.131	0.259
300mM imidazole fraction 1	2.573	1.620
300mM imidazole fraction 2	1.465	0.537
CAT control (negative)	0.185	0.174
BHC 15 100 μg/ml (positive)	2.305	0.689

*OD at 450nm

Epitope Mapping

Epitope mapping was undertaken by S. Tucker (Murex Diagnostics) with two anti-HCV monoclonals and a Pepscan kit from Cambridge Bioscience, Cambridge, UK. An HCV peptide sequence from a UK blood donor, (personnel communication, P. Highfield and colleagues at Murex Diagnostics) was synthesised on the pins.

RESULTS

Purification and Analysis of Expressed Core Protein

The 600 bp fragment (HCV core), which was amplified by semi-nested PCR, was cloned into pRCII. Subsequently, it was cloned into the pRSET vector and then expressed in *E. coli*. A nickel binding resin was used to bind specifically to the histidine tag on the expressed HCV core protein and to separate it from the *E. coli* proteins present after induction. Conditions for purification were optimised emperically. Initially, native and denaturing protocols were followed.

At least three times as much core protein was detected (by ELISA) when the cell pellet was re-suspended in a denaturing buffer as opposed to a native buffer. A denaturing protocol was selected, using imidazole rather than low pH, to release the Ni bound protein from the column. The eluate was then dialysed in a re-naturing buffer. The 300 mM imidazole fraction contained the majority of the core activity (Table I).

SDS page and Western blotting were carried out on four samples (Fig. 1a, b), purified HCV core, HCV lysate before purification, BHC15 core fusion protein, and CAT expressed protein. The same volume and concentration of sample was used in SDS PAGE as in Western blotting. Although Ni chromatography, as judged by SDS PAGE,

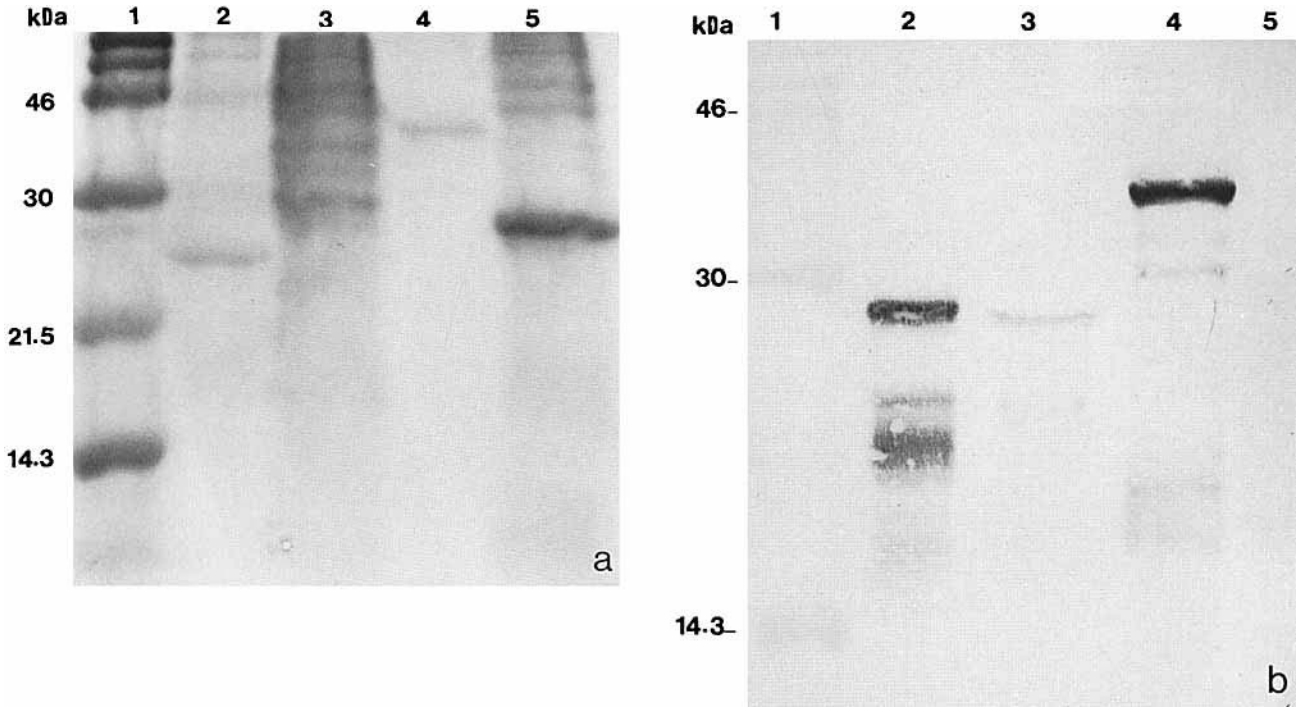

Fig. 1. **a:** SDS PAGE analysis of HCV core, BHC15, and CAT proteins on a 15% acrylamide gel. Lane 1: Molecular weight standards; lane 2: IMAC purified and concentrated core; lane 3: core lysate before purification; lane 4: BHC 15 core fusion protein; lane 5: pRSET expressed CAT protein. **b:** Western blot analysis of HCV core proteins run in Figure 1a SDS PAGE. Lane 1: Molecular weight standards (rainbow markers, faint); lane 2: IMAC purified and concentrated core; lane 3: core lysate before purification; lane 4: BHC 15 core fusion protein; lane 5: pRSET expressed CAT protein.

resulted in a much purer HCV product, some non-specific protein still remained (Fig. 1a). The HCV core specific protein was detected as a series of proteins (27kDa, a smear at 22kDa and 16kDa) with the anti-HCV human conjugate in Western blotting (Fig. 1b). A weak band was observed with the unpurified sample and the control BHC 15 fusion protein showed a major band at 36kDa with two weaker bands below. These smaller fragments observed with the *E. coli* expressed core and BHC 15 core fusion protein were presumably degradation products of the larger 27kDa protein. The CAT protein which gave a strong band in SDS PAGE (28 kDa) showed no reaction in Western blotting.

Identification and Characterisation of Anti-HCV Core Monoclonals

From the fusion of two HCV core immunised mouse spleens, 960 hybridomas were screened using the BHC15 ELISA and 78 of these gave OD_{450} values of above 0.5. Six hybridomas were selected and characterised as a representative cross-section of the positive hybridomas obtained (Table II).

Five of the six hybridomas gave a positive result with the Murex HCV ELISA. 2D2 and 3D7 were positive in immunofluorescence with BHC15 core fusion protein expressed in baculovirus cells (Fig. 2). (No staining was seen with a control anti-HIV *gag* monoclonal). Immunohistology was carried out on HCV-positive frozen liver sections using 2D2 and 6G11 SNF. Between 1 and 5% of hepatocytes showed cytoplasmic staining with the 2D2 monoclonal whereas 6G11 showed no specific staining. 2D2 and 3C4 SNF were tested in the HCV pepscan assay and neither mapped conclusively with the pins. Although none of the monoclonals reacted in the peptide based Inno-lia HCV III blotting assay, two of the monoclonals, 2D2 and 3D7, reacted with the core component of the Murex HCV RIBA assay (Fig. 4). When Western blotting was carried out on BHC15 core fusion protein and the core protein (Table II), all the SNF containing antibodies bound the 36kDa protein series of the BHC15 protein. However, with the core protein, 4F4 and 4G8 failed to react with the 22kDa and 16kDa lower molecular weight core proteins. 6G11 and 3C4 reacted with the 27kDa and 22kDa proteins but the 3C4 reactivity was very weak. Only 2D2 and 3D7 showed intense staining with all the 27, 22 and 16kDa protein series.

Competition of Monoclonals With Themselves and Six Human Anti-HCV Sera

Four of the six monoclonals were able to inhibit themselves from binding to BHC15 core fusion protein coated solid-phase (Table III). Surprisingly 6G11 and 4G8 failed to do so. From the patterns of inhibition monoclonals 2D2 and 3D7 appeared to be very similar in specificity (Ia). 4F4 and 4G8 (Ib) showed limited cross-blocking of the 2D2 and 3D7 but their epitope may be slightly different, which would explain the negative immunofluorescence and HCV Murex RIBA results, as compared

TABLE II. Characterisation of HCV Core Monoclonal Antibodies

Monoclonal antibody supernatant fluid	ELISA		Isotype	Murex HCV RIBA	Western blotting		IF BHC15	IH Liver section
	BHC 15Ag	Murex HCV			BHC 15	core series		
2D2	2.432 δ	2.461	IgG1	core pos	pos	27a* 22 16	pos	pos
3C4	0.555	0.660	IgM	neg	pos	27 22	neg	Nd
3D7	1.926	2.288	IgG1	core pos	pos	27 22 16	pos	Nd
4F4	2.072	2.263	IgG2a	neg	pos	27	neg	Nd
4G8	2.670	2.372	IgM	neg	pos	27	neg	Nd
6G11	2.466	0.299	IgA	neg	pos	27 22	neg	neg

IF, Immunofluorescence staining; IH, Immunohistological staining; Nd, Not done; δ, OD value at 450 nm. a*Protein size recognized in kDa.

TABLE III. Competition of Monoclonals With Themselves and Five Anti-HCV Sera

		Percentage inhibition						
		Labelled antibodies						
Unlabelled antibodies		291h	2D2m	3D7m	4G8m	4F4m	3C4m	6G11m
	Concentration		Group Ia	Group Ia	Group Ib	Group Ib	Group IIa	Group IIb
2D2m	5 μg/50 μl	+	+++	+++	+	++	−	+
3D7m		−	+++	+++	+	++	−	−
4G8m		−	−	−	−	−	−	+
4F4m		−	++	+++	−	++	−	++
3C4m		−	−	−	−	+	++	++
6G11m		−	−	−	−	−	−	+
G7H9m (neg)		−	−	−	−	−	−	−
344h	Neat	+++	+	+	−	+	++	+++
984h		+++	−	−	−	−	+	−
573h		Nd	−	−	−	−	+	+
291h		Nd	−	+	−	−	+	++
Ah		Nd	−	−	−	−	−	−
NHS		−	−	−	−	−	−	−

+++, 100–75% inhibition; ++, 74–50% inhibition; + 49–25% inhibition; − 24–0% inhibition; NHS, Normal human serum, -ve for HCV; Nd, Not done; m, Mouse monoclonal; h, Human HCV +ve serum, all strong ++++ C22 core on Murex RIBA apart from A = + only.

with 2D2 and 3D7. 3C4 (IIa) showed a completely different pattern of inhibition from these four, but its epitope may be similar to 6G11 (IIb). Both 3C4 and 6G11 were inhibited from binding by anti-HCV human sera to a similar extent and more so than the others (2D2, 3D7, 4F4, 4G8).

To summarise, four epitopes were recognised by the six monoclonals. The Ia and b epitopes may overlap with each other as with the IIa and IIb. Although no strong inhibition of human sera 291 was seen with any of the monoclonals (Table III) this is not surprising as there must be several other core epitopes in the human polyclonal anti-HCV serum which are able to bind the antibody onto the solid-phase to give a signal. 6G11 and 3C4 (II) were both able to be blocked from binding by several different human sera showing that they recognised an HCV epitope which is present in some anti-HCV core positive sera.

DISCUSSION

In this study we generated HCV core protein which was used to raise monoclonal antibodies in mice. The core protein of HCV is an important target for humoral and cellular immune responses, and thus reagents which help clarify the interaction between host and virus may be useful.

The whole full length core sequence, from 335 to 912 bp, was cloned into *E. coli* and the protein expressed with a six histidine tag at the N-terminus. Three factors influenced the selection of *E. coli* for expression of the HCV core. Firstly, the system has been used successfully to express HCV core proteins [Nasoff et al., 1991; Handschuh and Caselman, 1995; Milton et al., 1995]. Secondly, high levels of protein are expressed compared to eukaryotic systems and thirdly, purification of the *E. coli* expressed protein, especially when a histidine tag

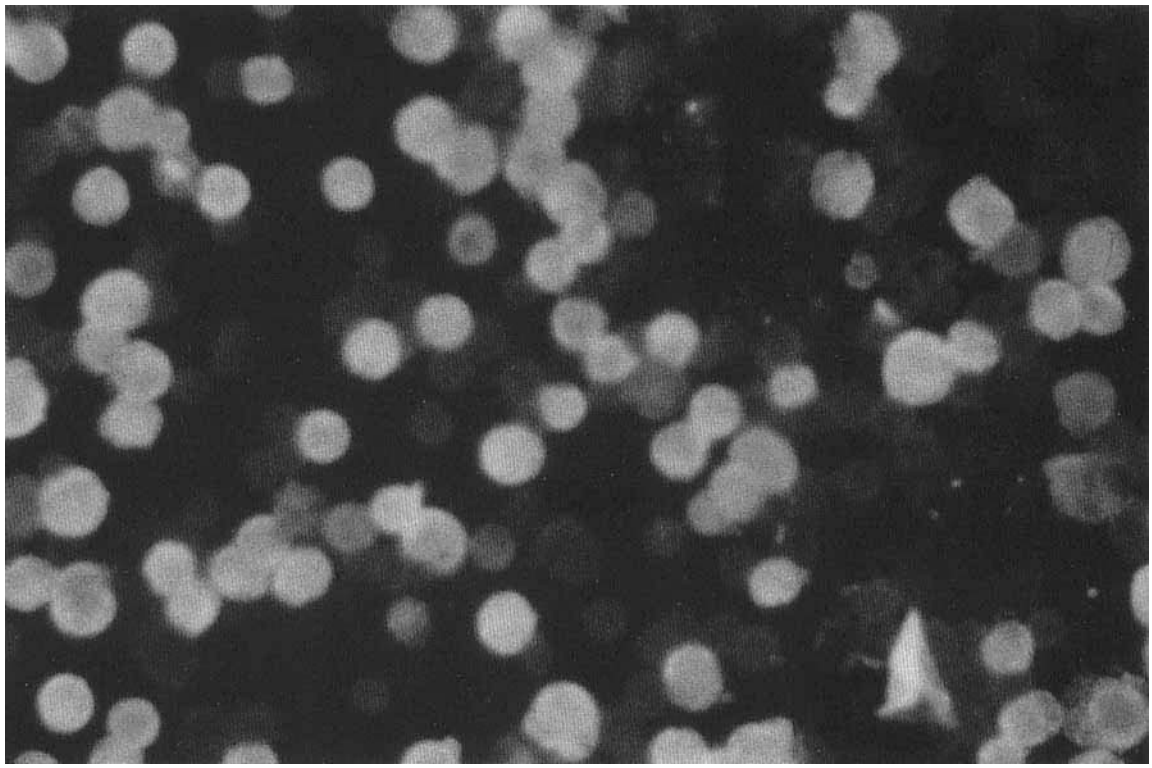

Fig. 2. Immunofluorescence of baculovirus cells expressing BHC15 core fusion protein. Baculovirus negative cells were mixed with an equal volume of cells expressing BHC 15 and then monoclonal antibody (2D2) followed by an anti-mouse fluorescein conjugate was used to detect HCV core activity.

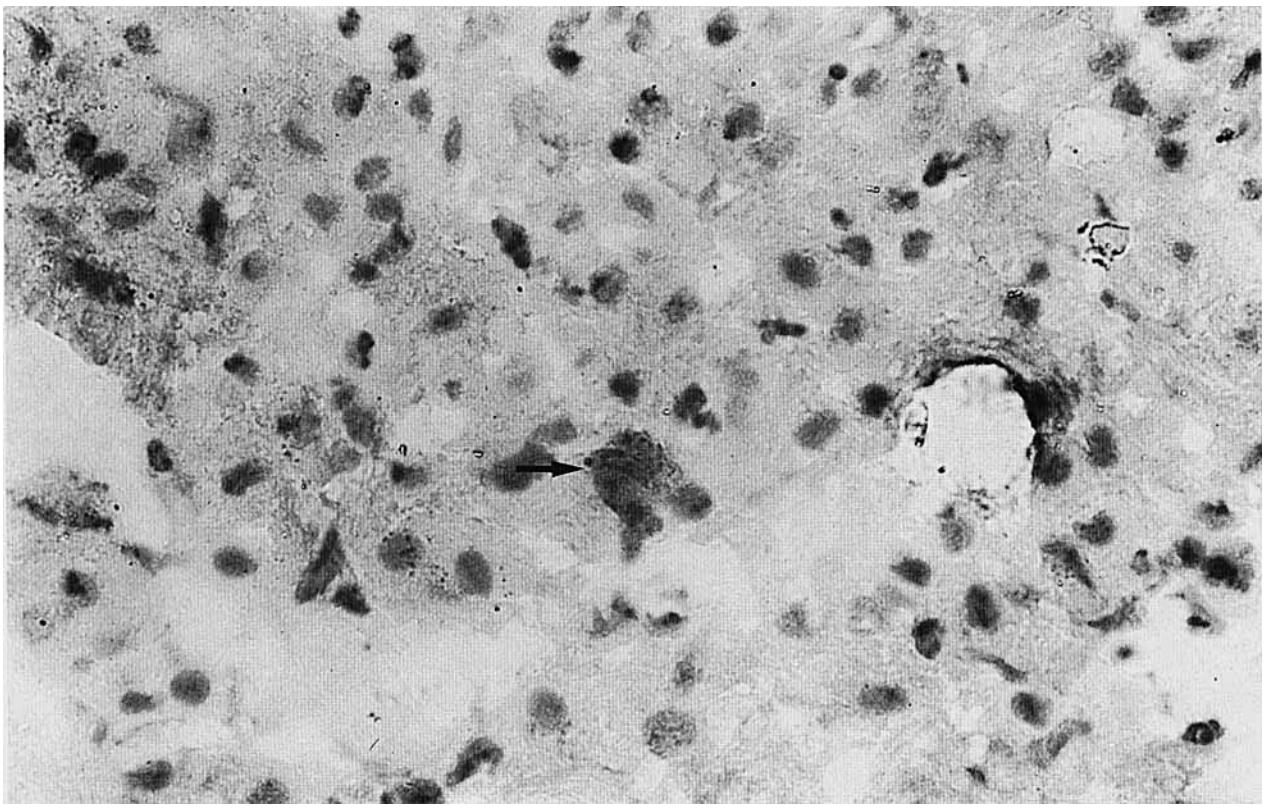

Fig. 3. Immunohistochemical staining of HCV infected liver. Frozen liver sections were prepared as described in the methods section and stained with monoclonal antibody 2D2. The arrow indicates core specific staining in cytoplasm (pink). The nuclei were counterstained blue.

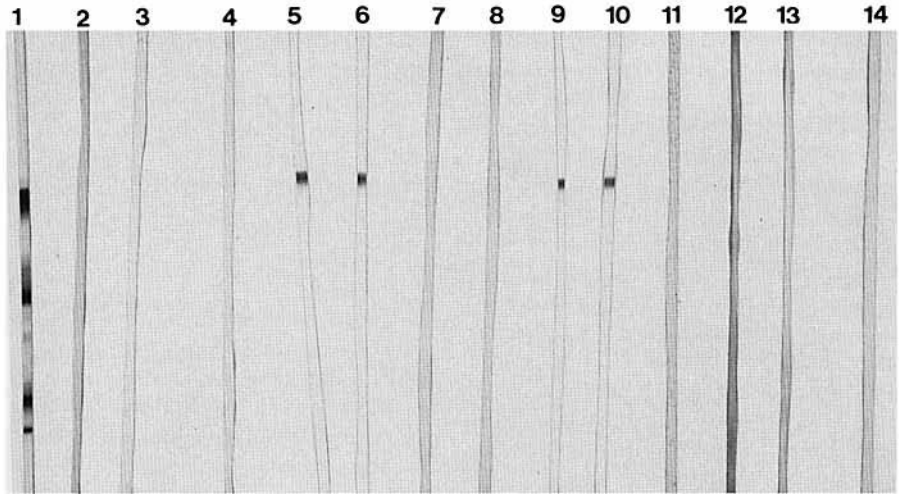

Fig. 4. Murex HCV RIBA results using kit controls and HCV-positive monoclonals (SNF, neat). Lane 1: Kit positive control (top band is core specific); lane 2: kit negative control; lane 3: mouse serum from immunised mice used for fusion; lane 4: HCV negative monoclonal, 3D3; lanes 5 and 6: 2D2; lanes 7 and 8: 3C4; lanes 9 and 10: 3D7; lane 11: 4F4; lanes 12 and 13: 4G8; lane 14: 6G11.

is attached is relatively straightward [Hoffmann and Roeder, 1991; Jankneht et al., 1991].

The C-terminus of the HCV core protein is hydrophobic and therefore it probably does not contain many B cell epitopes. However, it has been shown to be important in retaining the protein in the cytoplasm [Ravaggi et al., 1994]. Truncation of the core protein around amino acid 123, which removes the hydrophobic sequence, allows the protein to relocate to the nucleus [Lanford et al., 1993; Suzuki et al., 1995]. Thus by expressing the complete core sequence, it was anticipated that the expressed core would be located in the cytoplasm.

The core protein was very insoluble and therefore denaturing buffers (8M urea) were used to solubilise the protein in the cell lysate. A high molarity (300 mM imidazole), was used to elute the bound tagged core protein from the column by displacing the tagged protein from the column. Analysis of the eluted material, after it was allowed to refold in a non-denaturing buffer, showed it was able to bind in the ELISA to the human anti-HCV conjugate 291 (Table I). The presence of the hydrophobic C-terminus may have reduced the expression levels and the solubility of the core protein [Nasoff et al., 1991; Ravaggi et al., 1994; Handschuh and Caselmann, 1995; Suzuki et al., 1995]. This could explain why the expressed protein could only be seen in SDS PAGE after IMAC purification and concentration (Fig. 1a).

The predicted size of the HCV core protein is 22kDa [Hijikata et al., 1991]. In our study the 577 bp core sequence (193 amino acids) was attached at the N-terminus to the histidine tag and an enterokinase cleavage signal (108 bp). This should give a protein of 229 amino acids which translates to a protein of 26kDa. A protein of approximately 27kDa was seen in SDS PAGE and Western blotting (Fig. 1a, b). Handschuh and Caselmann [1995] found using *E. coli* and a histidine tagged N-terminal core protein, a core protein which migrated faster than expected. Proteolysis of the core protein in vaccinia virus and *E. coli* expression systems has been described [Selby et al., 1993; Milton et al., 1995] and a series of lower molecular weight products, as seen in Figure 1b, were observed. The removal of the hydrophobic C-terminus may have occurred to produce these smaller fragments since most of the antigenic determinants are still left, i.e., detected in Western blotting, after removal the C-terminus [Suzuki et al., 1995].

The semi-purified and refolded core (27 kDa and smaller) was able to induce anti-HCV core antibodies in mice. The core protein was highly immunogenic, as demonstrated by the number of "positive" colonies obtained. The monoclonal antibodies generated were identified using the BHC15 core fusion protein (amino acids 1–141). This screening system was chosen to avoid anti-*E. coli* monoclonals being selected. Clones were chosen which gave a range of results in ELISA. This procedure helps to select antibodies which recognise different epitopes [Ferns et al., 1991] and the six monoclonals chosen fell roughly into four different groups.

The Inno-lia HCV III assay failed to recognise any of the monoclonals, probably because this assay is based on core peptides, which detect only linear core epitopes. The Murex RIBA kit, however, which uses expressed proteins, gave a positive result with only 2D2 and 3D7 antibodies (Fig. 4). The 2D2 epitope was present in liver sections from HCV infected patients although at low levels (between 1–5% positive cells in cytoplasm), Figure 3, 2D2 was also positive in immunofluorescence with the BHC fusion protein. This monoclonal must recognise a "native" core epitope as well as a recombinant core epitope, and thus at least one of the exposed epitopes in the liver cells (immuno-

histology) and in the immunofluorescence stained BHC core protein must be similar.

After IMAC the degraded proteins observed in Western blotting probably still contained the N-terminal histidine tag which allowed its purification. Cleaving the protein from 27kDa to 16kDa did not remove the 2D2 and 3D7 epitope. Interpretation of core Western blot reactivities for 2D2 and 3D7 suggest their epitope may be located towards the N-terminus of the core protein and this epitope either resists SDS PAGE and 8M urea treatment, or is able to reform under these conditions. Evidence for this epitope being conformational was shown by the failure of the rabbit anti-HCV core antibody, (raised against amino acids 29–45 and used in the ELISA for detecting core antigen) to block binding of the monoclonals (results not shown) and the negative results obtained with the pepscan and the Inno-lia HCV III kit. These two monoclonals probably recognise a conformational epitope in the first half of the core sequence (1–140 amino acids). Both 4G8 and 4F4 gave partial inhibition of 2D2 and 3D7 (Table III). This blocking may represent steric hinderance or the epitopes these monoclonals recognise may overlap. Both antibodies were negative in BHC15 immunofluorescence and Murex RIBA and recognised only the whole 27kDa core protein.

As has been reported by Nasoff et al. [1991], Salberg et al. [1992, 1994], Goeser et al. [1994], and Gonzalez-Peralta et al. [1994] the epitopes for the core protein appear to be located in the first 110 amino acids. At least six linear epitopes and a major conformational epitope reside within this region. Two reports of HCV-positive liver cell immunohistology [Gonzalez-Peralta et al., 1994; Yap et al., 1994] describe the use of monoclonals which recognised linear epitopes located between amino acids 26–45 and 5–19 respectively. Monoclonals recognising amino acids 39–74 failed to detect HCV by immunofluorescence [Gonalez-Peralta et al., 1994]. Competition assays using both their monoclonals and ours may show if the epitope which 2D2 and 3D7 (group Ia) recognise is located within the first 45 amino acids.

Turning to the location of the group II monoclonal epitopes, both 3C4 and 6G11 failed to detect the lower molecular weight degradation 16 kDa protein in Western blot. Thus this epitope may be located between 105 amino acids (end of 16 kDa protein) and 160 amino acids (end of 22 kDa protein). The cross-competition data confirmed that they recognise a different epitope to the group I monoclonals. The epitope is probably conformational because 3C4 and 6G11 failed to react in the Inno-lia HCV III assay and in pepscan (6G11 was not tested). They also showed higher levels in inhibition with anti-HCV core positive sera than the group I monoclonals (Table III). This data suggests that more group II epitopes may be expressed on the HCV core protein circulating in the blood. In contrast, only group Ia epitopes only were detected in liver cells infected with HCV. Inhibition studies with other HCV core-positive sera should confirm this finding.

Further mapping of the monoclonals could perhaps include competition studies with previously characterised monoclonals and 3D7, 4G8, 4F4, and 6G11 monoclonals tested in pepscan. These reagents, especially 2D2 and 3D7, should prove to be useful in monitoring the expression of HCV in tissue culture and in fixed liver cell immunohistology (formalin-fixed sections have not been tested). They may also provide sensitive specific antibodies for developing an HCV core antigen detection assay.

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